Mutation of the Multiple Endocrine Neoplasia Type 1 Gene in Nonfamilial, Malignant Tumors of the Endocrine Pancreas

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Abstract
Endocrine pancreatic tumors are rare neoplasms that occur sporadically or as part of a multiple endocrine neoplasia type 1 (MEN1) syndrome. Germ-line mutations of the MENI gene, located at 11q13, have been demonstrated in MEN1 kindreds, and loss of heterozygosity (LOH) on 11q13 together with somatic MEN1 mutations have been detected in 20% of nonfamilial parathyroid tumors. Here, we examine 11 non-MEN1 malignant tumors of the endocrine pancreas, 9 nonfunctioning tumors, and 2 glucagonomas. LOH of at least one informative locus on 11q13 was found in 70% of the tumors. Three tumors displayed somatic mutations of the MEN1 gene together with LOH on 11q13, whereas the corresponding germ-line DNA was normal. These findings support the hypothesis that MEN1 gene mutations contribute to the tumorgenesis of nonfamilial, malignant endocrine pancreatic tumors.

Introduction
Endocrine pancreatic tumors are rare benign or malignant neoplasms that occur sporadically or as part of a MEN1 syndrome. MEN1 is an autosomal, dominantly inherited disorder with tumors of the endocrine pancreas, as well as lesions in the parathyroid, the pituitary, and other tissues (1). Tumors of the endocrine pancreas are often associated with well-recognized clinical syndromes due to the excess secretion of specific hormones, or they may be clinically nonfunctioning. The MENI gene, a putative tumor suppressor gene, has been mapped to chromosome 11q13, and tumors of the MENI patients, as well as nonfamilial tumors of the parathyroid, the endocrine pancreas, and the pituitary, have shown LOH for this locus (2-4). Recently, the MENI gene was identified by positional cloning, and over 40 different germ-line mutations have been found in investigated MEN1 kindreds (5-7). Thus far, no correlation between genotype and phenotype has been found. Moreover, loss of heterozygosity on 11q13, together with somatic mutations in the MENI gene, were subsequently detected in approximately 20% of nonfamilial parathyroid tumors (8). In this study, we have performed LOH analysis on 11q13 and sequence analysis of the MENI gene on non-MEN1 malignant tumors of the endocrine pancreas.

Materials and Methods
Eleven patients with malignant tumors of the endocrine pancreas were studied, 9 with nonfunctioning tumors (no clinical symptoms of endocrine activity), and 2 with classical glucagonomas. None of them displayed a family history of endocrine tumors or signs of MEN1 at thorough biochemical and radiological investigation (1). All tumors displayed morphological signs of malignancy by means of invasive growth pattern, large size, and multiple hormonal staining, and nine had metastasized to liver and/or lymph nodes at the time of surgery. Tumor DNA was extracted from cryosections by standard procedures after microdissection to avoid gross contamination by nontumor cells. Paired germ-line DNA was extracted from leukocytes or normal pancreatic tissue.

LOH Screening. Six microsatellite markers within the 11q13 region (D11S480, INT-2, D11S527, D11S916, D11S906, and Fcer1) were chosen for the analysis (9, 10). PCR reactions contained 20 ng of template DNA, 1-12 pmol of each primer (one of which was end-labeled with 32P), 100 μM each deoxynucleotide triphosphate, 1× reaction buffer (Life Technologies, Inc.), 1.5 mM MgCl2, and 0.2 unit Taq DNA polymerase (Life Technologies, Inc.) in a final volume of 10 μl. The reactions were performed in a GeneAmp 9600 thermal cycler (Perkin-Elmer Corp.) as follows: denaturation at 95°C for 4 min, followed by 27 cycles of annealing at 55°C for 30 s, extension at 72°C for 30 s, and denaturation at 95°C for 30 s. Then, a 4-min final extension at 72°C was carried out. Reactions were separated on a 4.5% denaturing polyacrylamide gel, and signals were quantified by Phosphorimager analysis (Molecular Dynamics). Complete loss or more than 70% reduction of one of the two alleles present in heterozygous individuals was considered LOH.

Sequence Analysis. PCR using published oligonucleotide primers flanking exons 2-10 of the MEN1 gene and tumor DNA of all patients was performed (7). The efficiency and stringency of the PCR was controlled by resolving the products on agarose gels. In case of low yield or stringency, reamplification was performed after gel purification. Both DNA strands of the amplified products were subjected to semiautomated sequencing on ABI 373A using the ABI Prism Dye Terminator Cycle Sequencing Ready Reaction kit with AmpliTaq DNA Polymerase, FS (Perkin-Elmer Corp.). In cases with suspected mutations, the analysis was repeated, and the paired germ-line DNA was sequenced as well.

Results and Discussion
LOH of at least one informative locus on 11q13 was found in 7 of 10 (70%) nonfamilial malignant tumors of the endocrine pancreas (Table 1). Both glucagonomas and one of the nonfunctioning tumors showed mutation of the MEN1 gene: one missense (L89R; Fig. 1) and two nonsense (E392Stop and R415Stop; Table 1). The corresponding germ-line DNA was unaltered. All of these three tumors displayed LOH on 11q13, making the tumor cells hemi- or homozygous for the mutant allele. In addition, two previously described polymorphisms (6) were identified, S145S (AGC/AGT) and D418D (GAC/GAT), with prevalence of 21 and 36% of sequenced alleles, respectively (n = 14). The germ-line DNA of case 5 with the somatic mutation R415Stop in the tumor DNA exhibited heterozygosity for the polymorphic locus D418D (GAC/GAT). Sequence analysis of the tumor DNA showed the R415Stop mutation linked to the allele with the GAC polymorphism, whereas the other allele of that polymorphic

Received 10/13/97; accepted 12/19/97.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1This work was supported by grants from the Swedish Medical Research Council, the Swedish Cancer Society, the Torsten and Ragnar Söderbergs Research Foundation, and Lions Fund for Cancer Research. Primers for LOH analysis were provided by the Dept. of Clinical Genetics, Uppsala University supported by the Council of the Nordic Ministers and the Swedish Medical Research Council.
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4The abbreviations used are: MEN1, multiple endocrine neoplasia type 1; LOH, loss of heterozygosity.

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Table 1 Clinical characteristics of cases with malignant tumors of the endocrine pancreas, relation to LOH, and sequence analysis of MEN1 exons 2–10

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Type</th>
<th>Tumor size (cm)</th>
<th>Metastasis</th>
<th>LOH&lt;sup&gt;a&lt;/sup&gt; 11q13</th>
<th>Mutation&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>44</td>
<td>M</td>
<td>Glucagonoma</td>
<td>5</td>
<td>Liver</td>
<td>+</td>
<td>E329Stop</td>
</tr>
<tr>
<td>2</td>
<td>53</td>
<td>M</td>
<td>Glucagonoma</td>
<td>3</td>
<td>Regional</td>
<td>+</td>
<td>L89R</td>
</tr>
<tr>
<td>3</td>
<td>57</td>
<td>M</td>
<td>Nonfunctioning</td>
<td>Unknown</td>
<td>Liver</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>65</td>
<td>F</td>
<td>Nonfunctioning</td>
<td>8</td>
<td>Regional</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>59</td>
<td>F</td>
<td>Nonfunctioning</td>
<td>6</td>
<td>Liver</td>
<td>+</td>
<td>R415Stop</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
<td>F</td>
<td>Nonfunctioning</td>
<td>5</td>
<td>Regional</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>53</td>
<td>M</td>
<td>Nonfunctioning</td>
<td>12</td>
<td>Liver</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>53</td>
<td>M</td>
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<td>6.5</td>
<td>None</td>
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<td>0</td>
</tr>
<tr>
<td>9</td>
<td>63</td>
<td>F</td>
<td>Nonfunctioning</td>
<td>8</td>
<td>None</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
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<td>F</td>
<td>Nonfunctioning</td>
<td>3.5</td>
<td>Regional</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>51</td>
<td>M</td>
<td>Nonfunctioning</td>
<td>2</td>
<td>Liver</td>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> +, 70% reduction or more was considered as LOH; -, no LOH; ND, not determined.

<sup>b</sup> 0, no mutations found.

locus was not seen. This shows that deletions detected by the LOH analysis includes the MEN1 gene, making this tumor hemizygous for the mutant allele.

The function of the MEN1 gene product is to date unknown, and the impact of the presently identified somatic mutations thus remains unclear. However, the two identified nonsense mutations can be predicted to create a truncated MEN1 gene product, possibly interfering with the putative tumor suppressor function of menin (5). The R415Stop mutation that we found in a malignant nonfunctioning endocrine pancreatic tumor has been found previously as a germ-line mutation in a MEN1 kindred, contributing to the inherited risk of tumor development (7). This particular family expresses clinical features suggestive of anticipation, and the frequency of nonfunctioning endocrine pancreatic tumors is prominent (11). The question whether some genotype-phenotype correlation exists for the R415Stop mutation, with future clinical implications, remains to be established. The third point mutation (L89R) implies substitution of the hydrophobic amino acid leucine for the positively charged arginine, which may interfere with the function of the protein. This latter mutation is located within the large exon 2, where several mutations have been found in MEN1 individuals and in one sporadic parathyroid tumor (7, 8). The fact that all tumors with mutations also displayed LOH on 11q13 supports the hypothesis that menin acts as tumor suppressor in a subset of these lesions.

Molecular mechanisms behind endocrine pancreatic tumor development are poorly understood. Previous studies of such tumors have shown LOH on chromosomes 11q13 and 3p in 19–45% and 33%, respectively (12–15). Our demonstration of LOH in 70% is consistent with a higher incidence of somatic events in the malignant subset of the nonfamilial endocrine pancreatic tumors. Twenty-seven % of the examined specimens showed homozygous mutations of the MEN1 gene, which consequently seems to be no requisite for the malignant transformation per se. On the other hand both glucagonomas, which represent a generally aggressive phenotype, displayed mutations. The findings support the hypothesis that mutations in the MEN1 gene contribute to tumorigenesis in some of the nonfamilial, malignant endocrine pancreatic tumors. Further characterization of the genetic alterations responsible for the heterogeneous endocrine syndromes and the variable degrees of malignant behavior in these neoplasms is warranted.

References

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